REVIEW ARTICLE

Collagen as Bioink for Bioprinting: A Comprehensive Review

Egor Olegovich Osidak[1,2]*, Vadim Igorevich Kozhukhov[2,3], Mariya Sergeevna Osidak[1], Sergey Petrovich Domogatsky[2,3]

[1]Imtek Ltd., 3rd Cherepkovskaya 15A, Moscow, Russia

[2]Gamaleya Research Institute of Epidemiology and Microbiology Federal State Budgetary Institution, Ministry of Health of the Russian Federation, Gamalei 18, Moscow, Russia

[3]Russian Cardiology Research and Production Center Federal State Budgetary Institution, Ministry of Health of the Russian Federation, 3 Cherepkovskaya 15A, Moscow, Russia

Abstract: Biomaterials made using collagen are successfully used as a three-dimensional (3D) substrate for cell culture and considered to be promising scaffolds for creating artificial tissues. An important task that arises for engineering such materials is the simulation of physical and morphological properties of tissues, which must be restored or replaced. Modern additive technologies, including 3D bioprinting, can be applied to successfully solve this task. This review provides the latest evidence on advances of 3D bioprinting with collagen in the field of tissue engineering. It contains modern approaches for printing pure collagen bioinks consisting only of collagen and cells, as well as the obtained results from the use of pure collagen bioinks in different fields of tissue engineering.

Keywords: Collagen, Three-dimensional bioprinting, Tissue engineering, Cell-laden hydrogels

***Corresponding Author:** Egor Olegovich Osidak, Gamaleya Research Institute of Epidemiology and Microbiology Federal State Budgetary Institution, Ministry of Health of the Russian Federation, Gamalei 18, Moscow, Russia; OsidakEgor_egorosidak@gmail.com

***Received*:** March 02, 2020; ***Accepted*:** March 16, 2020; ***Published Online*:** April 21, 2020

(This article belongs to the *Special Section: Bioprinting in Russia*)

Citation: Osidak EO, Kozhukhov VI, Osidak MS, *et al*., 2020, Collagen as Bioink for Bioprinting: A Comprehensive Review. *Int J Bioprint*, 6(3): 270. DOI: 10.18063/ijb.v6i3.270.

1 Introduction

For the past few years, additive technologies, including the technology of three-dimensional (3D) bioprinting, have emerged into a rapidly developing tissue engineering sphere[1]. These technologies allow creating layer-by-layer assembled structures with a specific pore size and porosity that promotes the restoration of defects of soft or hard tissues. Another indisputable advantage of the 3D bioprinting is that it allows creating personalized implants for the specific needs of a patient, taking the individual features of the patient into account at the same time[2]. Moreover, the use of this technology allows building complex structures that are already colonized with cells at the moment of bioprinting. Cell-laden hydrogels, which are also called bioinks, are used to create such structures[3].

Synthetic polymers, such as poly(ethylene glycol), as well as native proteins, such as collagen, can be used as a structural basis for such hydrogels[3,4]. Collagen-containing hydrogels are currently the most popular cell scaffold and material for tissue engineering, especially if working with cells is

intended[5]. Nevertheless, the most important thing here is that materials created using 3D bioprinting and collagen have very high chances of clinical success in the future, because collagen biomaterials have been already been actively and successfully used in clinical practice for a long time. This is possible due to the unique properties of collagen – biocompatibility and low immunogenicity[6,7]. However, low immunogenicity of 3D constructs can be achieved only with the use of high purity collagen solutions without potential immunogenic admixtures[6]. Thus, in this review, "collagen" will be meant as a purified protein obtained through extraction from collagen-containing tissues and not a decellularized extracellular matrix of any tissue or organ containing a large amount of collagen[8]. The main barrier that prevents the use of decellularized materials is immunological rejection, which significantly limits the possibility of clinical use of such materials.

Therefore, the purpose of this review is to conduct a comprehensive study of the use of collagen-based bioinks for 3D bioprinting in various fields of tissue engineering. The review covers topics such as general limitations and advantages of collagen and collagen-based bioinks used in different areas and the main approaches for collagen-based bioinks 3D printing.

2 Pure collagen bioink: Printability aspects

As it was already noted before, soft biomaterials loaded with living cells are called bioinks[3]. The basis of collagen bioink is a collagen hydrogel, physical properties of which represent its printability. The majority of collagen hydrogels are produced from type I collagen, which makes up around 90% of the protein mass in the connective tissues of mammals[9]. Type I collagen belongs to the group of fibril-forming collagens and consists of three alpha-helices that form a triple-helical structure[9,10]. Under physiological conditions (neutral pH and 37°C), collagen molecules start to self-organize into fibrils, and collagen solution forms a hydrogel. The printability of collagen bioink depends on the kinetics of this process – the higher the speed, the higher is printing accuracy.

The majority of existing studies on 3D printing and bioprinting using collagen specify the main problem with collagen bioink – its low mechanical properties[3,11]. All these studies were carried out using collagen solutions of low concentration – usually, not more than 5 mg/ml and rarely, 10 mg/ml[12]. This problem refers to not only in 3D bioprinting but also other sections of tissue engineering. More than 90% of known studies were carried out using collagen hydrogels prepared from solutions with not more than 10 mg/ml collagen[5].

One of the possible approaches to overcome this limitation is the use of supportive hydrogels. When using a supportive hydrogel for 3D bioprinting with a collagen bioink, the whole process occurs inside of the secondary hydrogel (e.g., gelatin slurry), which in turn acts as temporary thermo-reversible support (FRESH technique – freeform reversible embedding of suspended hydrogels)[13-15]. On the one hand, this method allows printing complex structures using collagen solutions of low concentrations with a polymerization period of 40 – 60 min. On the other hand, gelatin from the supportive hydrogel can diffuse inside the bioink during the polymerization period. This, in turn, will lead to a final construct that potentially contains gelatin. The effect of the remaining FRESH gelatin in a final construct at *in vivo* implantation is not fully studied.

Another approach to compensate for the low mechanical properties of collagen hydrogels was proposed by Diamantides *et al*.[16]. According to their study, the best way to improve the printability of collagen bioinks is to increase the storage modulus of the ink before extrusion. This strategy was described more accurately by Osidak *et al*.[17], it was shown that collagen bioinks with a much greater storage modulus than loss modulus are suitable for direct extrusion bioprinting.

The storage modulus of collagen solution depends on the concentration of NaCl in the solution[18], its temperature[19], and on collagen concentration[17,19,20]. The most effective method to increase the storage modulus is to increase the collagen concentration in a solution[17]. Such highly concentrated collagen solutions of 80 mg/ml named Viscoll Bioink (viscous collagen

bioink solution) are available on the market. When mixed with a mammal cell suspension in a cultural medium and then heated to 37°C, Viscoll bioink quickly forms a stable cell-laden hydrogel. The survival rate of NIH 3T3 cells as a part of rigid collagen hydrogels was approximately 90% after printing and after a week of *in vitro* cultivation. Unfortunately, this is the only data on the behavior of cells during cultivation inside rigid 3D collagen hydrogels that are currently available.

3 Tissue engineering applications of collagen- based bioinks

Due to the prevalence of collagen-based bioinks with a low protein concentration usage in various fields of tissue engineering, collagen is mixed with various materials to improve the manufacturing process and the final characteristics of the printed construct[21,22]. There are only few studies, where collagen bioinks were used as a pure substance without any additives. These works are listed below.

Currently, there are two general methods for creating tissue-engineering designs – *in vitro* bioprinting and *in situ* bioprinting. In the case of *in vitro* bioprinting, the printing of design is carried out in the laboratory environment. After printing, the design is either implanted into a laboratory animal or cultivated for a specific period for cell behavior study. In the case of *in situ* bioprinting, printing is carried out directly onto the defective area of a laboratory animal.

3.1 Skin

Koch *et al*.[23] in their work have printed a construct with the use of laser-assisted bioprinter onto the surface of a supportive scaffold – decellularized dermal matrix (Matriderm). The printing process was carried out in two stages – 20 layers of fibroblast (murine NIH 3T3) were applied onto the surface, which was followed by 20 layers of keratinocyte (human HaCaT), embedded into collagen hydrogel (3 mg/ml). As a result, it was shown that a bi-layered construct that generates dermis and epidermis has been successfully created. After 10 days of cell cultivation inside of the construct, the presence of Connexin 43 in the epidermis, which showed the ability to form gap junctions, was detected. In another study of Koch *et al*.[24], similar bi-layered constructs were created in *in vitro* conditions and then implanted *in vivo*, employing the dorsal skin-fold chamber in nude mice. It was found that fibroblasts can migrate into a supportive scaffold. Moreover, it was noted that the presence of several blood vessels in the wound bed could be observed after 11 days of transplantation.

Shi *et al*.[25] have printed six-layered cellular structures using extrusion-based bioprinter. They used three types of cells: Human melanocytes (HEM), HaCat, and human dermal fibroblasts (HDF). As a material for bioink, they used a mixture of GelMA and collagen. In addition, I-2959 photoinitiator and tyrosinases were added to the obtained mixture. The biocompatibility of created designs was evaluated *in vitro* and *in vivo* through implantation of these structures without cells into a full-thickness wound model of Sprague-Dawley rat. The viability of these three cell lines during 14 days of cultivation was above 90%. *In vivo* tests have shown that healing rates of the wound can be accelerated when treated with the tyrosinase doped bioinks.

Another study worth noting was made up by Yoon *et al*.[12]. To create 3D skin substitutes, they used pure (single-component) collagen bioinks. Primary human epidermal keratinocytes (HEK) and HDF were used to fabricate cell-laden 3D scaffolds. Cell-laden 3D scaffolds were created through extrusion bioprinting and were composed of four layers. The top-level contained keratinocytes and the other three layers had fibroblasts. According to the results of the study, cell-laden 3D scaffolds in a 1×1 cm^2 full-thickness excision mouse model have successfully demonstrated their efficiency. After 1 week, the damaged skin almost completely and clearly regenerated. The hair follicles on the wound bed also regenerated almost perfectly.

In the work of Skardal *et al*.[26], amniotic fluid-derived stem cells (AFSC) and mesenchymal stem cells (MSC) were separately suspended in the fibrinogen/collagen solution. They used a bioprinter to directly print two layers of a fibrin-collagen gel by depositing a layer of thrombin, a layer of fibrinogen/collagen, another layer of thrombin, another layer of fibrinogen/collagen,

and the final layer of thrombin onto a full-thickness skin wound (2 × 2 cm^2). In 2 weeks, AFS-treated mice showed an average of 3% of unclosed wounds, whereas MSC-treated wounds showed an average of 2%. These values were significantly lower than those of mice treated with gel only, which had an average of 13% of unclosed wounds.

Further development of this result was continued by Albanna *et al*.[27], where excisional wounds were bioprinted with layered autologous dermal fibroblasts and epidermal keratinocytes in a fibrinogen/collagen carrier (25 mg/ml fibrinogen, and 1.1 mg/ml collagen) in two different models: Murine full-thickness wound model (3 × 2.5 cm) and porcine full-thickness wound model (10 × 10 cm). The obtained results showed a rapid wound closure, reduced contraction, and accelerated re-epithelialization.

3.2 Bone and cartilage

Native bone tissues can withstand heavy loads. Therefore, 3D printed structures, ideally must possess the same characteristics. In this case, to strengthen 3D bioprinted structures, composite materials are being actively used nowadays, for example, a mixture of collagen with various types of bioceramics[28-31].

Kim *et al*.[29] have introduced bioceramic-based cell-printing technique and a cell-laden ceramic structure. Using 3D bioprinting technology, they created a cell-laden scaffold using α-tricalcium phosphate (α-TCP) type I collagen and MC3T3-E1 cells. First, they have printed a porous layer consisting of micro-sized α-TCP/collagen struts without cells, and then a cell-laden collagen bioink was printed onto it. This procedure was repeated several times to form a 3D porous cell-laden ceramic scaffold. The elastic modulus of the α-TCP/collagen scaffold was 550 kPa. However, this value is much lower than the elastic modulus of a real trabecular bone (around 20 MPa)[32]. Nevertheless, it was shown that the designed scaffold demonstrated good cellular activities, including metabolic activity and mineralization.

In the other work of Kim and Kim[28], β-TCP, type I collagen and MC3T3-E1 cells were used as a bioink, and Genipin was used as a crosslinking agent. With new materials, the elastic modulus of printed structures was 5.94 MPa. The *in vitro* evaluation of cellular responses (viability and proliferation) was comparable to results obtained in the pure cell-laden collagen.

One of the earliest studies on cartilage bioprinting using pure collagen bioinks was carried out in 2016 when primary meniscal fibrochondrocytes and high-density collagen hydrogels (from 10 to 20 mg/ml) were bioprinted[20]. In that study, the influence of collagen on several parameters, including geometric fidelity, cell viability, and mechanical properties of printed constructs, was evaluated. The concentration of collagen gel had no impact on cell viability, whereas the compressive modulus of printed gels increased linearly with an increase in collagen concentration. With the highest printable concentration, the elastic modulus of the printed structure reached 30 kPa. These structures maintained cell viability and their geometric fidelity for 10 days while being stored in a culture medium. The geometric accuracy of structures, printed with 15 mg/ml and 17.5 mg/ml collagen solutions, was at 74 – 78%.

Shim *et al*.[33] have printed a construct for osteochondral tissue regeneration in the rabbit knee joint. Pure collagen bio-ink that consisted of atelocollagen, human turbinate-derived mesenchymal stromal cells (hTMSCs), and recombinant human bone morphogenetic protein-2 (rhBMP-2), was printed into a preprinted polycaprolactone (PCL) scaffold. The prepared cylinder-shaped construct was 5 mm in diameter and 5 mm in height, with a "subchondral bone layer" (PCL, atelocollagen, hTMSCs, and rhBMP-2) of 4 mm in thickness, and "superficial cartilage layer" (Cucurbit[6]uril, hTMSCs, and TGF-β) of 1 mm in thickness. This construct was *in vivo* implanted onto the defective part of the rabbit knee joint. Eight weeks later, it was shown that the construct possessed a capability for osteochondral regeneration. The adjacent native cartilage maintained its structure without any signs of degeneration. The newly regenerated cartilage tissues smoothly integrated themselves with ends of the host cartilage tissue. The immunohistochemical analysis for collagen type

II (COL-II) and COL-X expression indicated that zonal cartilage regeneration was reached.

An alternative approach was proposed by Yang *et al*[34]. In this work, to prepare the bioink, collagen (15 mg/ml) was mixed with alginate (sodium alginate SA/COL). Then, primary chondrocytes isolated from articular cartilage of new-born rats were added to the mixture. As a comparison, two types of other bioinks were used: Bioink made from agarose (AG) and bioink made from a mixture of alginate and AG (SA/AG). When comparing SA/COL with SA/AG and SA, the proliferation and survival of chondrocytes were significantly promoted in the case of SA/COL bioinks. The expression of specific gene markers of cartilage, including Sox9, Acan, and Col2a1, was also significantly higher in SA/COL group.

3.3 Cardiovascular tissues

In general, the research work on bioprinting for cardiovascular tissue regeneration is focused on myocardium, heart valves, and vasculature[35].

Maxson *et al*.[14] have demonstrated the potential of the use of the highly concentrated type I collagen hydrogel for the heart valve bioprinting. In addition to collagen, the bioink contained rat MSCs. With this bioink, Maxson *et al*. managed to print collagen disks of 1 mm in thickness and 28 mm in diameter onto a FRESH slurry. After slurry removal, the printed structures were subcutaneously implanted into rats. The implanted samples were extracted at 2, 4, 8, and 12 months with the subsequent study on their mechanical properties, evaluation of cell infiltration, and determination of levels of specific inflation markers expression. The profile of stress-strain curves of the bioprinted aortic heart valve scaffolds indicated that scaffolds have transitioned through phases of resorption, synthesis, stabilization, ultimately, and remodeling.

At the resorption stage (2 – 4 weeks), the mechanical properties of implanted scaffolds were reduced. Moreover, the increased expression of CD3 biomarker from acute inflammation was also noted during this period.

At the synthesis stage (4 – 8 weeks), the mechanical properties of implanted scaffolds began to increase. Gross encapsulation of the implanted scaffold, which additionally indicates an inflammatory reaction, was also noted.The concentration of CD3 biomarker (that is an indication of an inflammatory process) was reduced compared with the first stage. However, the gross encapsulation of the implanted scaffold shows that the inflammation process is still presented.

At the stabilizing stage (8 – 12 weeks), peak values of elastin, vimentin, and alpha-SMA production were noted. This indicates the active deposition of collagen by infiltrated cells and the strengthening of the extracellular matrix in the bioprinted scaffold. However, compared with the previous stage, there was no significant increase in the mechanical properties of the implanted material.

This work of Maxson *et al*. is of particular interest due to the demonstrated recellularization potential of a bioprinted aortic heart valve scaffold from a highly concentrated type I collagen hydrogel. Furthermore, an increased level of the CD3 marker expression in experimental animals may be associated with the presence of residual gelatin from FRESH slurry and allogeneic rat cells in the construct.

Despite concerns of inducing a systemic immune response, it is worth noting that the indisputable advantage of FRESH technology is the ability to 3D print the human heart components at various scales from capillaries to the full organ with a high degree of accuracy. For example, Lee *et al*.[15] have printed a simplified model of a small coronary artery–scale linear tube from COL for perfusion with a custom-designed pulsatile perfusion system. The linear tube had an inner diameter of 1.4 mm and a wall thickness of ~300 μm. C2C12 cells within a collagen gel were cast around the printed collagen tube. Only in case of active perfusion through the collagen structure, cells remained alive in their entirety. Next, they printed the left ventricle of the heart using human stem cell-derived cardiomyocytes. The ventricle was designed as an ellipsoidal shell with inner and outer walls of collagen and a central core region which contained cells. Cardiac ventricles printed with human cardiomyocytes showed synchronized contractions, directional action

potential propagation, and wall thickening of up to 14% during peak systole.

Summarizing the above, the data[14,15] have demonstrated the effectiveness of the use of highly concentrated type I collagen hydrogel as a main material of bioinks for cardiovascular tissue regeneration.

3.4 Liver

A variety of 3D printing techniques are used for liver tissue engineering[36]. The general purpose of such works, along with a recreation of a complex microarchitecture and cell diversity, is a development of sustainable *in vitro* models of the liver for drug testing and pathology study.

As an example, Shim *et al*.[37] have successfully developed a hybrid scaffold consisting of PCL and MC3T3-E1-laden collagen hydrogel. The scaffold was prepared using a multi-head deposition system, followed by primary hepatocyte seeding to create a patterned 3D coculture. In the proposed method, a tough supporting PCL construct was used to maintain the specific 3D form of the printed structure. However, it should be noted that the mechanical properties of such structures differ from the mechanical properties of the liver tissues significantly, and the problem of the printed structure stabilization for a long-term *in vitro* cell cultivation remains open. To solve this problem, Mazzocchi *et al*.[38] have proposed to use a mixture of methacrylated type-I collagen and hyaluronic acid as a structural basis for bioink. Into this mixture, they introduced primary human hepatocytes. After that, the resulting structure was crosslinked using UV irradiation. The printed cell-laden constructs were incubated in a culture medium for 15 days. The functionality of hepatocytes was evaluated on the 6th day of cultivation by exposure constructs to acetaminophen (APAP) and hepatic toxicant. Levels of cell expression of albumin, urea, and lactic acid dehydrogenase (LDH) into the culture medium were also evaluated. A pronounced decrease in the levels of albumin and urea expression was found on the 9th day of cultivation in the APAP treated group. This reduction continued until day 15. In contrast with the untreated group, these parameters were stable during the whole period of cultivation. Levels of alpha-glutathione S-transferases and detoxification protein increased at day 9 (3 days after APAP addition) but subsequently decreased by day 12, which was more likely due to cell death. APAP treated constructs demonstrated decreasing LDH activity, again, likely due to toxicity related cell death. Untreated conditions maintained steady LDH levels.

3.5 Nervous system models and regeneration

Collagen, as a material, is widely used in nerve regeneration[39,40]. The neurite growth is more pronounced in collagen hydrogels, prepared using mildly concentrated collagen solutions[41,42]. Therefore, such collagen solutions are used for 3D printing more often[43,44] in this field of tissue engineering, whereas there are only few studies that report the use of highly concentrated collagen solution[45].

In 2009, Lee *et al*.[43] have proposed a direct cell printing technique to pattern neural cells in a 3D multilayered collagen hydrogel. First, they printed a layer of collagen hydrogel to create a scaffold for cells. Next, rat embryonic neurons and astrocytes were printed onto the existing layer. The process was repeated layer-by-layer to create 3D cell hydrogel composites. This study demonstrated the ability of microvalve printing to create a pattern of various cells in a single construct.

In another work[44], microvalve printing was used to create a layered 3D neural stem cell (NSC)-laden hydrogel collagen construct. Next to collagen hydrogel, a thrombin crosslinked fibrin gel was printed. The fibrin gel acted as a depo releasing the vascular endothelial growth factor (VEGF) for 3 days. Cells in the collagen construct migrated to the VEGF-releasing fibrin gel. During the experiment, the increased proliferation and increased branched morphology with neurite projections were observed. In control samples, cells did not show any signs of proliferation or migration (where fibrin without VEGF or VEGF was printed directly into collagen).

Chen *et al*.[45] have created 3D bioprinted collagen-heparin sulfate scaffolds. To promote axonal regeneration and functional recovery

Table 1. Collagen-based bioinks for different tissue-engineering applications.

Bioink		Cross-link	Supportive Scaffold	*In vitro* /*In vivo*	Bioprinting technology	Ref.
Materials	**Cells**					
Skin						
Collagen I type	HEK and HDF	pH and temperature	-	Both	Extrusion-based bioprinting	[12]
Collagen I type	NIH 3T3 and HaCaT	pH and temperature	Matriderm	*In vitro*	Laser-assisted bioprinting	[23]
Collagen I type	NIH 3T3 and HaCaT	pH and temperature	Matriderm	Both	Laser-assisted biopriting	[24]
Collagen I type and GelMa	HEM, HaCaT, and HDF	pH and temperature	-	Both	Extrusion-based bioprinting	[25]
Collagen I type and fibrin	AFSCs and MSCs	Thrombin	-	*In vivo*	Ink-jet in situ bioprinting	[26]
Collagen I type and fibrin	dermal fibroblasts and epidermal keratinocytes	Thrombin	-	*In vivo*	Ink-jet in situ bioprinting	[27]
Bone and cartilage						
Collagen I type and β-TCP	MC3T3-E1 and hASCs	Genipin	-	*In vitro*	Extrusion-based bioprinting	[28]
Collagen I type	MC3T3-E1	Tannic cid	α-TCP/ collagen	*In vitro*	Extrusion-based bioprinting	[29]
Collagen I type	fibrochondrocytes	pH and temperature	-	*In vitro*	Extrusion-based bioprinting	[20]
Collagen I type	hTMSCs	pH and temperature	PCL	Both	Extrusion-based bioprinting	[33]
Collagen I type and alginate	chondrocytes	CaCl2	-	*In vitro*	Extrusion-based bioprinting	[34]
Cardiovascular tissues						
Collagen I type	MSCs	pH and temperature	FRESH	*In vivo*	Extrusion-based bioprinting	[14]
Collagen I type	C2C12 and hESC-CMs	pH and temperature	FRESH	*In vitro*	Extrusion-based bioprinting	[15]
Liver tissues						
Collagen I type	MC3T3-E1	pH and temperature	PCL	*In vitro*	Extrusion-based bioprinting	[37]
Methacrylated collagen type I and hyaluronic acid	hepatocytes	UV light	-	*In vitro*	Extrusion-based bioprinting	[38]
Nervous system						
Collagen I type	neurons and astrocytes*	pH and temperature	-	*In vitro*	Microvalve printing	[43]
Collagen I type	C17.2*	pH and temperature	-	*In vitro*	Microvalve printing	[44]
Collagen I type and heparin sulfate	NSCs*	UV light	-	Both	Extrusion-based bioprinting	[45]
Cornea						
Methacrylated collagen type I and alginate	hCSKs	CaCl2	FRESH	*In vitro*	Extrusion-based bioprinting	[47]
Collagen I type and agarose	hCSKs	pH and temperature	-	*In vitro*	Electromagnetic microvalve bioprinting	[48]

*In this study, cells were not a part of a bioink–hey were seeded onto the surface of the 3D printed scaffold. HEK: Human epidermal keratinocytes; HDF: Human dermal fibroblasts; AFSC: Amniotic fluid-derived stem cells; MSCs: Mesenchymal stromal cells; hTMSCs: Human turbinate-derived mesenchymal stromal cells; NSC: Neural stem cell; UV: Ultraviolet; α-TCP: α-tricalcium phosphate; PCL: Polycaprolactone; FRESH: Freeform reversible embedding of suspended hydrogels

from spinal cord injury, they cultivated NSC on the surface of scaffolds. Next, scaffolds were implanted into transsection lesions in T10 of the spinal cord in rats. Two months after, a significant recovery of locomotor functions was observed.

3.6 Cornea

Cornea bioprinting is one of the new approaches in tissue engineering[46]. The extracellular matrix of the native cornea consists of almost 90% of I type collagen. This is why bioinks for artificial cornea also must contain collagen. The bioprinting of cornea through the extrusion method allows controlling the thickness and geometrical properties of a printed structure. For example, Isaacson *et al*.[47] have printed a corneal like cell-laden structure. As a bioink, they used a mixture of SA and methacrylated type I collagen with encapsulated corneal keratocytes. Cell survival in the printed structures during 7-day cultivation was at a high level. Similar results on cell survival were obtained in AG, collagen, and corneal stromal keratocytes bioinks[48].

These studies show that it will be possible to create an artificial cornea in the future. However, *in vitro* experiments do not give sufficient information for it. For this case, more studies that imply 3D bioprinting of different versions of the artificial cornea will have to be tested *in vivo*.

4 Concluding remarks and future perspectives

For the last few years, there was significant progress in 3D bioprinting and adaptation of collagen solutions to the needs of this technology. Without any doubt, the combination of collagen-based bioink and 3D bioprinting has great potential in the manufacture of artificial organs and tissues for tissue engineering and regenerative medicine. **Table 1** provides a list of existing variants of collagen-based bioinks that could be used for such purposes. However, the development in this direction is very slow. It is mostly connected with the absence of easily-accessible collagen bioinks which would correspond to requirements of the "perfect" bioink. The applicability of collagen for 3D bioprinting depends on collagen concentration in a solution. Only high concentrations of collagen (more than 20 mg/ml) in single-component collagen bioinks allow increasing the accuracy of printing. At present, there are only a few commercially available concentrated collagen bioinks – Lifeink® (35 mg/ml, Advanced Biomatrix, USA) and Viscoll® (80 mg/ml, Imtek, Russia). One of the distinctive characteristics of these bioinks is the possibility to add not only cells but also any components of the extracellular matrix to their composition. This allows to bioprint an artificial cell-laden matrix, which can be required by a researcher to solve a specific problem.

Despite the absence of sufficient data on the behavior of mammalian cells in dense collagen hydrogels, a large number of scientists are concerned that cells will inevitably collapse in dense collagen hydrogels during cultivation. However, primary tests[17] have debunked those concerns. It was found that fibroblasts retain their high viability in high-density collagen gels (up to 40 mg/ml). These results lead the way to some new studies devoted to the behavior of cells in high-density collagen hydrogels, their proliferation and migration activity, differentiation, functionality retention, as well as the creation of various scaffolds using 3D printing technology with their subsequent colonization with cells in various fields of tissue engineering.

Acknowledgments

This work was funded by the Foundation for Assistance to Small Innovative Enterprises (FASIE), Project no. 44700.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

1. Xia Z, Jin S, Ye K, 2018, Tissue and organ 3D bioprinting. *SLAS Technol*, 23:301–314. DOI: 10.1177/2472630318760515.
2. Nagarajan N, Dupret-Bories A, Karabulut E, *et al*., 2018, Enabling personalized implant and controllable biosystem development through 3D printing. *Biotechnol Adv*, 36:521–

533. DOI: 10.1016/j.biotechadv.2018.02.004.

3. Hospodiuk M, Dey M, Sosnoski D, *et al*., 2017, The bioink: A comprehensive review on bioprintable materials. *Biotechnol Adv*, 35:217-239. DOI: 10.1016/j.biotechadv.2016.12.006.
4. Kloxin AM, Kloxin CJ, Bowman CN, *et al*., 2010, Mechanical properties of cellularly responsive hydrogels and their experimental determination. *Adv Mater*, 22:3484–94. DOI: 10.1002/adma.200904179.
5. Antoine EE, Vlachos PP, Rylander MN, 2014, Review of collagen I hydrogels for bioengineered tissue microenvironments: Characterization of mechanics, structure, and transport. *Tissue Eng Part B Rev*, 20:683–96. DOI: 10.1089/ten.teb.2014.0086.
6. Lynn AK, Yannas IV, Bonfield W, 2004, Antigenicity and immunogenicity of collagen. *J Biomed Mater Res B App Biomater*, 71:343–54. DOI: 10.1002/jbm.b.30096.
7. Parenteau-Bareil R, Gauvin R, Berthod F, 2010, Collagen-based biomaterials for tissue engineering applications. *Materials* (*Basel*), 3:1863–87. DOI: 10.3390/ma3031863.
8. Sriya Y, Shibu C, Ashis KB, *et al*., 2019, Tissue-Specific Bioink from Xenogeneic Sources for 3D Bioprinting of Tissue Constructs, in Xenotransplantation-comprehensive Study, Shuji Miyagawa, IntechOpen, Available from: https://www.intechopen.com/books/xenotransplantation-comprehensive-study/tissue-specific-bioink-from-xenogeneic-sources-for-3d-bioprinting-of-tissue-constructs. DOI: 10.5772/intechopen.89695.
9. Gelse K, Pöschl E, Aigner T, 2003, Collagens--structure, function, and biosynthesis. *Adv Drug Deliv Rev*, 55:1531–46. DOI: 10.1016/j.addr.2003.08.002.
10. Ricard-Blum S, 2011, The collagen family. *Cold Spring Harb Perspect Biol*, 3:a004978. DOI: 10.1101/cshperspect.a004978.
11. Włodarczyk-Biegun MK, Del Campo A, 2017, 3D bioprinting of structural proteins. *Biomaterials*, 134:180–201. DOI: 10.1016/j.biomaterials.2017.04.019.
12. Yoon H, Lee JS, Yim H, *et al*., 2016, Development of cell-laden 3D scaffolds for efficient engineered skin substitutes by collagen gelation. *RSC Adv*, 6:21439–47. DOI: 10.1039/c5ra19532b.
13. Hinton TJ, Jallerat Q, Palchesko RN, *et al*., 2015, Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels. *Sci Adv*, 1:e1500758. DOI: 10.1126/sciadv.1500758.
14. Maxson EL, Young MD, Noble C, *et al*., 2019, *In vivo* remodeling of a 3D-bioprinted tissue engineered heart valve scaffold. *Bioprinting*, 16:e00059. DOI: 10.1016/j.bprint.2019.e00059.
15. Lee A, Hudson AR, Shiwarski DJ, *et al*., 2019, 3D bioprinting of collagen to rebuild components of the human heart. *Science*, 365:482–87. DOI: 10.1126/science.aav9051.
16. Diamantides N, Wang L, Pruiksma T, *et al*., 2017, Correlating rheological properties and printability of collagen bioinks: The effects of riboflavin photocrosslinking and pH. *Biofabrication*, 9:034102. DOI: 10.1088/1758-5090/aa780f.
17. Osidak EO, Karalkin PA, Osidak MS, *et al*., 2019, Viscoll collagen solution as a novel bioink for direct 3D bioprinting. *J Mater Sci Mater Med*, 30:31. DOI: 10.1007/s10856-019-6233-y.
18. Lian D, Jiheng L, Conghu L, *et al*., 2013, Effects of NaCl on the rheological behavior of collagen solution, *Korea-Aust Rheol J*, 25:137–144. DOI: 10.1007/s13367-013-0014-9.
19. Lai G, Li Y, Li G, 2008, Effect of concentration and temperature on the rheological behavior of collagen solution. *Int J Biol Macromol*, 42:285–91.
20. Rhee S, Putzer JL, Mason BN, *et al*., 2016, 3D Bioprinting of spatially heterogeneous collagen constructs for cartilage tissue engineering. *ACS Biomater Sci Eng*, 2:1800–1805. DOI: 10.1021/acsbiomaterials.6b00288.
21. Lee H, Yang GH, Kim M, *et al*., 2018, Fabrication of micro/nanoporous collagen/dECM/silk-fibroin biocomposite scaffolds using a low temperature 3D printing process for bone tissue regeneration. *Mater Sci Eng C Mater Biol Appl*, 84:140-147. DOI: 10.1016/j.msec.2017.11.013.
22. Moncal KK, Ozbolat V, Datta P, *et al*., 2019, Thermally-controlled extrusion-based bioprinting of collagen. *J Mater Sci: Mater Med*, 30:55. DOI: 10.1007/s10856-019-6258-2.
23. Koch L, Deiwick A, Schlie S, *et al*., 2012, Skin tissue generation by laser cell printing. *Biotechnol Bioeng*, 109:1855–63.
24. Michael S, Sorg H, Peck CT, *et al*., 2013, Tissue engineered skin substitutes created by laser-assisted bioprinting form skin-like structures in the dorsal skin fold chamber in mice. *PLoS One*, 8:e57741. DOI: 10.1371/journal.pone.0057741.
25. Shi Y, Xing TL, Zhang HB, *et al*., 2018, Tyrosinase-doped bioink for 3D bioprinting of living skin constructs. *Biomed Mater*, 13:035008. DOI: 10.1088/1748-605x/aaa5b6.
26. Skardal A, Mack D, Kapetanovic E, *et al*., 2012, Bioprinted amniotic fluid-derived stem cells accelerate healing of large skin wounds. *Stem Cells Transl Med*, 11:792–802. DOI: 10.5966/sctm.2012-0088.
27. Albanna M, Binder KW, Murphy SV, *et al*., 2019, *In Situ* bioprinting of autologous skin cells accelerates wound healing of extensive excisional full-thickness wounds. *Sci*

Rep, 9:1856. DOI: 10.1038/s41598-018-38366-w.

28. Kim W, Kim G, 2019, Collagen/bioceramic-based composite bioink to fabricate a porous 3D hASCs-laden structure for bone tissue regeneration. *Biofabrication*, 12:015007. DOI: 10.1088/1758-5090/ab436d.
29. Kim WJ, Yun HS, Kim GH, 2017, An innovative cell-laden α-TCP/collagen scaffold fabricated using a two-step printing process for potential application in regenerating hard tissues. *Sci Rep*, 7:3181. DOI: 10.1038/s41598-017-03455-9.
30. Lin KF, He S, Song Y, *et al*., 2016, Low-temperature additive manufacturing of biomimic three-dimensional hydroxyapatite/collagen scaffolds for bone regeneration. *ACS Appl Mater Interfaces*, 8:6905–16.
31. Marques CF, Diogo GS, Pina S, *et al*., 2019, Collagen-based bioinks for hard tissue engineering applications: A comprehensive review. *J Mater Sci Mater Med*, 30:32. DOI: 10.1007/s10856-019-6234-x.
32. Mishra R, Basu B, Kumar A, 2019, Physical and cytocompatibility properties of bioactive glass-polyvinyl alcohol-sodium alginate biocomposite foams prepared via sol-gel processing for trabecular bone regeneration. *J Mater Sci*: *Mater Med*, 20:2493–500. DOI: 10.1007/s10856-009-3814-1.
33. Shim JH, Jang KM, Hahn SK, *et al*., 2016, Three-dimensional bioprinting of multilayered constructs containing human mesenchymal stromal cells for osteochondral tissue regeneration in the rabbit knee joint. *Biofabrication*, 8:014102. DOI: 10.1088/1758-5090/8/1/014102.
34. Yang X, Lu Z, Wu H, *et al*., 2018, Collagen-alginate as bioink for three-dimensional (3D) cell printing based cartilage tissue engineering. *Mater Sci Eng C Mater Biol Appl*, 83:195–201. DOI: 10.1016/j.msec.2017.09.002.
35. Cui H, Miao S, Esworthy T, *et al*, 2018, 3D bioprinting for cardiovascular regeneration and pharmacology. *Adv Drug Del Rev*, 132:252–269.
36. Lewis PL, Shah RN, 2016, 3D Printing for liver tissue engineering: Current approaches and future challenges. *Curr Transpl Rep*, 3:100–108. DOI: 10.1007/s40472-016-0084-y.
37. Shim JH, Kim JY, Park M, *et al*., 2011, Development of a hybrid scaffold with synthetic biomaterials and hydrogel using solid freeform fabrication technology. *Biofabrication*, 3:034102. DOI: 10.1088/1758-5082/3/3/034102.
38. Mazzocchi A, Devarasetty M, Huntwork R, *et al*., 2018, Optimization of collagen Type I-hyaluronan hybrid bioink for 3D bioprinted liver microenvironments. *Biofabrication*, 11:015003. DOI: 10.1088/1758-5090/aae543.
39. Klein S, Vykoukal J, Felthaus O, *et al*., 2016, Collagen Type I conduits for the regeneration of nerve defects. *Materials* (*Basel*), 9:219. DOI: 10.3390/ma9040219.
40. Madduri S, Feldman K, Tervoort T, *et al*., 2010, Collagen nerve conduits releasing the neurotrophic factors GDNF and NGF. *J Control Release*, 143:168-74. DOI: 10.1016/j.jconrel.2009.12.017.
41. O'Connor SM, Stenger DA, Shaffer KM, *et al*., 2000, Primary neural precursor cell expansion, differentiation and cytosolic Ca(2+) response in three-dimensional collagen gel. *J Neurosci Methods*, 102:187–95. DOI: 10.1016/s0165-0270(00)00303-4.
42. Labour MN, Vigier S, Lerner D, *et al*., 2016, 3D compartmented model to study the neurite-related toxicity of Aβ aggregates included in collagen gels of adaptable porosity. *Acta Biomater*, 37:38–49. DOI: 10.1016/j.actbio.2016.04.001.
43. Lee W, Pinckney J, Lee V, *et al*., 2009, Three-dimensional bioprinting of rat embryonic neural cells. *Neuroreport*, 20:798–803. https://doi.org/10.1097/wnr.0b013e32832b8be4.
44. Lee YB, Polio S, Lee W, *et al*., 2010, Bio-printing of collagen and VEGF-releasing fibrin gel scaffolds for neural stem cell culture. *Exp Neurol*, 223:645–52. DOI: 10.1016/j.expneurol.2010.02.014.
45. Chen C, Zhao ML, Zhang RK, *et al*., 2017, Collagen/heparin sulfate scaffolds fabricated by a 3D bioprinter improved mechanical properties and neurological function after spinal cord injury in rats. *J Biomed Mater Res A*, 105:1324–32. DOI: 10.1002/jbm.a.36011.
46. Zhang B, Xue Q, Li J, *et al*., 2019, 3D bioprinting for artificial cornea: Challenges and perspectives. *Med Eng Phys*, 71:68–78.
47. Isaacson A, Swioklo S, Connon CJ, 2018, 3D bioprinting of a corneal stroma equivalent. *Exp Eye Res*, 173:188–193. DOI: 10.1016/j.exer.2018.05.010.
48. Campos DD, Rohde M, Ross M, *et al*., 2019, Corneal bioprinting utilizing collagen-based bioinks and primary human keratocytes. *J Biomed Mater Res Part A*, 107:1945–53. DOI: 10.1002/jbm.a.36702.